MITIGATION OF RICIN CONTAMINATION IN SOILS: SORPTION AND DEGRADATION

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INTRODUCTION

Ricin is a highly toxic protein isolated from castor beans. Ricin is a weapon of choice for terrorists because large quantities of castor beans can be grown and the toxin readily separated from the beans.

Soils contain a variety of inorganic minerals, organic matter and microorganisms. Soil inorganic minerals and organic matter are known to effectively sorb a wide variety of compounds, such as pesticides and other potential contaminants. Soil microorganisms are known to degrade a variety of organics, such as petroleum and pesticides. Because clay mineralogy, organic matter content and microbial populations differ in soils, it is important to identify minerals that strongly sorb ricin and characterize microorganisms that can effectively degrade the protein. The minerals, organic matter and microorganisms in some soils might be more effective in sorption and degradation of ricin than other soils.

The objectives of this research were to examine the capacity of soil constituents and microbes to retain and degrade ricin that was released into the environment. To achieve these objectives, we examined: (1) the ricin contents of soils in formerly and currently cropped castor fields; (2) the functional and taxonomic diversity of microbes in castor versus cotton field soils; (3) the capacity of soil microbes to degrade ricin; and (4) the capacity of soil minerals and other materials to sorb ricin.

MATERIALS AND METHODS

Plots of castor (Fig. 1) were grown at a Texas Tech University farm during and two years prior to this study. Within season ricin concentrations were determined in castor plots that were center pivot or furrow irrigated. An enzyme-linked immunosorbent assay (ELISA) was used to measure ricin concentrations in supernatants after sorption experiments and in extracts of castor field soils. Standard ricin, rabbit anti-ricin antibody, enzyme-labeled goat anti-rabbit antibody and other ELISA reagents were obtained from Sigma-Aldrich Chemical Company.

Microbial functional diversity was measured in soils cultivated with castor and cotton based on the ability of the microbes to use 95 different carbon compounds on a microtiter plate (BIOLOG^{TM}). Soil samples from a cotton field were selected to act as controls. Taxonomic

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diversity was also measured in the castor and cotton field soils by identification of the dominant fungi and bacteria.

Batch sorption isotherms were prepared by adding ricin solutions to weighed samples in centrifuge tubes. Equilibrium ricin concentrations were determined on the supernatants after 8 hours shaking, centrifugation and filtration. Similarly, ricin in physiological saline extracts of soils was measured in the extracts after centrifugation and filtration. Properties of the samples used in batch sorption experiments are presented in Table 1. Nitrogen surface areas were measured using a single point method. Cation exchange capacities were obtained from published values.

RESULTS AND DISCUSSION

We examined the ricin contents of soils in formerly and currently cropped castor fields. Ricin concentrations as determined by ELISA were below detection limits until seed maturation-December concentrations were 0.28 and 0.72 μg ricin/g soil for the furrow irrigated and the center pivot plots, respectively. These values possibly reflect the growth and seed production differences due to irrigation. Ricin concentrations in soils from a field where castor had been cultivated two years earlier contained 0-0.32 μg ricin/g soil. This suggests that ricin might be stable when present in the soil and is not subject to rapid degradation.

Fungal and bacterial functional diversity (Fig. 2) declined in soils after bean maturation. Fungal taxonomic diversity (Table 2) declined in soils cultivated with castor relative to fields cultivated with cotton. This might indicate sensitivity of certain fungal species to residual ricin in the soil. Fungi being eukaryotic are expected to be susceptible to ricin. Bacteria, which have a different ribosome structure from eukaryotes are resistant to ricin and therefore any observed changes in populations, cannot be directly ascribed to ricin levels. However, since bacteria may be dependant on fungi for degradation of certain components of soil organic matter, fluctuations in bacterial numbers might reflect fluctuations in fungal populations. Actinomycete concentrations as high as 30,000/g of soil identified in castor field soils might effectively decompose ricin. In vitro assays indicate that this group of bacteria was not effective at degrading ricin. On the other hand, tow other bacteria genera, *Pseudomonas* and *Erwinia* were observed to effectively degrade the protein in in vitro assays.

Ricin sorption isotherms using silicate clay minerals (Fig. 3) indicate effective sorption by 2:1 minerals, such as montmorillonite and illite. The montmorillonites sorbed up to 400 mg ricin/g and were the most effective sorbents. Figure 4 illustrates the interlayer region of 2:1 minerals, such as montmorillonite. The interlayer region contains exchangeable cations, such as Na^{+}, Ca^{+2}, Mg^{+2} and K^{+} that are replaceable. Kaolinite, a 1:1 silicate clay, did not effectively sorb ricin. In Fig. 5, iron oxides, humic acid, and calcite effectively sorbed ricin. Activated decolorizing carbon sorbed minor amounts of ricin, whereas, activated cocoanut charcoal, quartz, glass, and concrete (not shown) did not sorb measurable amounts of ricin.

In Fig. 6, pH clearly has a strong effect on ricin sorption by montmorillonite. Montmorillonite effectively sorbed ricin at pHs of 4 and 7, but not at pH 10. At pH 10, the ricin molecule is primarily anionic (negatively-charged) and coulombic repulsion would act to exclude it from the negatively-charged surfaces of montmorillonite.

Soils containing expandable clay minerals, such as montmorillonite, have a high swell/shrink potential because the interlayer expands when moist and collapses when dry. The soils with the highest swell/shrink potential (Fig. 7) should be effective ricin sorbents. In

contrast, highly permeable soils (Fig. 8) with coarser textures and less expandable clays should not be effective ricin sorbents.

Table 1. Properties of the materials used in the batch studies

Sample	Material	Source	BET N2 Surface Area m^2/g	CEC Cmol/kg
Ca-montmorillonite	Expanding 2:1 clay	Clay Min. Soc.	76	120
Na-montmorillonite	Expanding 2:1 clay	Clay Min. Soc.	27	76
Kaolinite	1:1 clay	Clay Min. Soc.	21	27
Sepiolite	Fibrous clay	Clay Min. Soc.	11	2
Palygorskite	Fibrous clay	Clay Min. Soc.	317	~15
Goethite	Fe-oxide	Synthetic	178	20
Ferrihydrite	Fe-oxide	USDA-ARS	31	-
Calcite powder	CaCO3	Fisher Scientific	227	-
Quartz sand	Quartz crystals	Hot Springs, AR	0.6	-
Ca-humate	Humic acid	Aldrich Chemical	-	-
Glass powder	Pyrex glass	Glass pipettes	-	~200
Concrete	Portland cement	Quickcrete	-	-
Decolorizing carbon	Activated carbon	Fisher Scientific	1013	-
Cocoanut carbon	Activated carbon	Fisher Scientific	1360	-

Table 2. Castor impacts on fungal taxonomic diversity: Total activity

Dominate fungi	
Castor field:	Fusarium (several species)
	Lower species richness
Cotton field:	Trichoderma
	Fusarium
	Sterile dark
	Higher species richness

Figure 1. Picture of castor plants growing on the Texas Tech University farm during the 2001growing season

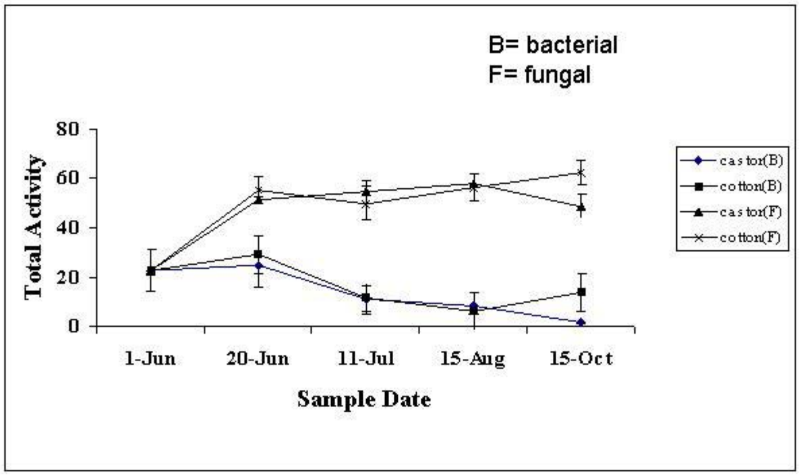

Figure 2. Functional diversity of fungi and bacteria during the 2001 growing season at the Texas Tech University farm.

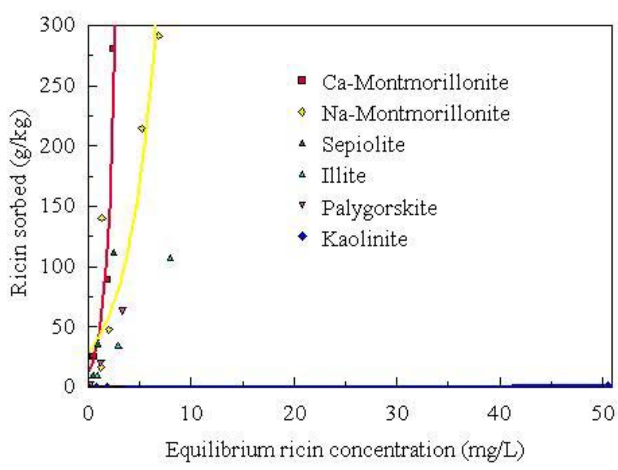

Figure 3. Ricin sorption isotherms.

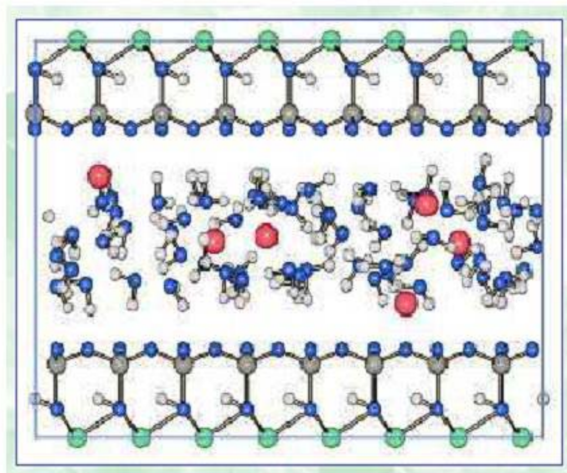

Figure 4. Interlayer regions of 2:1 clay minerals such as montmorillonite.

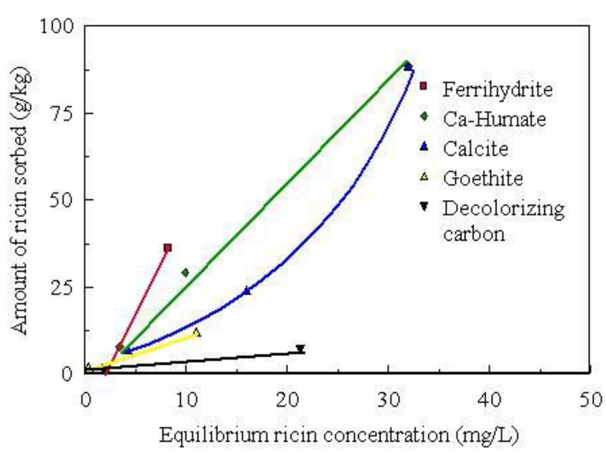

Figure 5. Ricin sorption on non-philloslicate materials.

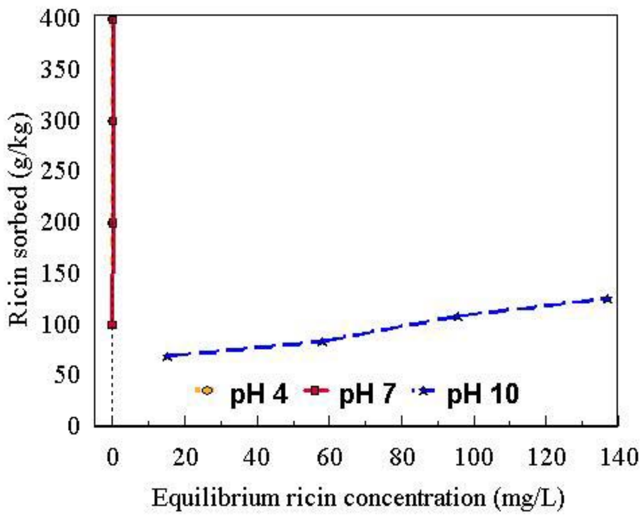

Figure 6. Ricin sorption as influenced by pH.

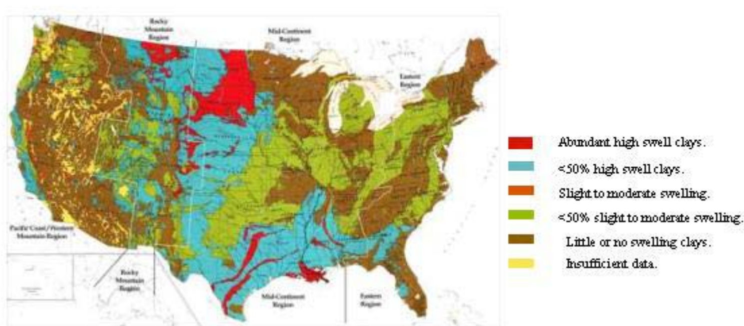

Figure 7. Figure depicting soils with high shrink-swell potential within the United States.

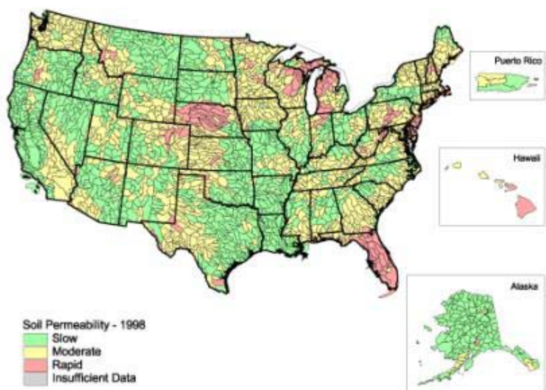

Figure 8. Figure depicting soils with high permeability within the United States.